THE NATURE OF VIRUSES*

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THIS paper is a brief summary of some facts and many opinions about the nature of the smaller viruses. This field of research has advanced in such spectacular fashion during the last ten years that virology has become a major division of microbiology with its own journal, symposia and professorships. In addition, research work with viruses has made important contributions to related fields such as immunology, genetics and cellular physiology. In discussing viruses it is important to distinguish between the smaller viruses such as those of poliomyelitis and influenza and the larger viruses such as those in the lymphogranuloma-psittacosis group. The latter viruses are not only structurally complex but demonstrate metabolic activities that are absent from the smaller viruses.

The virus of poliomyelitis is spherical in shape and about 28 mμ in diameter.[1] For comparison the mass of the psittacosis virus is about 4000 times greater and the mass of small bacteria is 20,000 times greater. The polio viruses appear to consist only of protein and ribose nucleic acid. The nucleic acid seems to be enclosed within a protective protein membrane because it is completely resistant to the enzyme ribonuclease. The polio viruses possess no detectable metabolism and they are so small (particle weight about six million times that of the hydrogen atom) that they cannot contain more than about 100 enzyme molecules per virus particle even if all of the proteins were functional enzymes. These viruses are completely dependent on the host cell for the raw materials, the energy and the synthetic machinery needed to produce themselves and for this reason they are obligate intracellular parasites. The virus of poliomyelitis was obtained in crystalline form last year,[2] and is the first mammalian virus to be crystallized. One may

* Presented at the 29th Graduate Fortnight of The New York Academy of Medicine, October 25, 1956.
† This paper was written by Dr. Adams before his sudden death on October 17, 1956. It was read by F. E. Wassermann.

question whether such a minute and relatively simple particle should be classed as a living organism. However, as we shall see, these small viruses do demonstrate certain of the essential attributes of life.

One important property of viruses is the ability to cause the production of offspring in their own image—they possess genetic continuity from generation to generation. They multiply rapidly and produce millions of identical descendants in less than a day. Among these progeny are found a few individuals with altered hereditary properties, with mutant genes. Under appropriate conditions these mutant virus particles can be isolated in pure culture and their properties studied. In the case of the polio viruses mutations have been reported affecting host cell specificity, virulence, heat stability and colonial morphology in tissue culture. Apparently these mutations involve little or no change in the serological specificity of the virus, so that mutation of a virus to a more restricted host range or decreased virulence may not alter its antigenic properties when used as a vaccine. In these two properties, genetic continuity and mutability, the smaller viruses are typical of all living organisms.

Because of its importance in the next paper[3] it might be well to discuss current concepts of microbial genetics as applied to viruses. That viruses can vary spontaneously in their properties has been recognized for many years but the rules governing the process have been understood only recently. There is considerable evidence that the substance that transmits genetic information from one generation to the next is nucleic acid. That is, it is the viral nucleic acid that tells the infected host cell how to make more virus particles. The mechanism by which the nucleic acid determines the specific structure of protein molecules, such as enzymes and antigens, is one of the major unsolved problems of biology today. But whatever the mechanism may be, it must involve the specific chemical structure of the nucleic acid, the sequence of the purine and pyrimidine bases along the molecule.

There is considerable justification, both theoretical and experimental, for assuming that a mutation may be the result of the replacement of a single base in a nucleic acid molecule by a different purine or pyrimidine. According to this picture genetic continuity is the result of the tendency of reproducing organisms to make exact replicas of their nucleic acid molecules, and mutations are the result of accidental errors in making the copies. It seems probable that the overall

frequency of such errors is rather large, perhaps one error in every ten virus particles, and that most of them are lethal to the organism. However, each particular kind of mutation must involve a specific replacement at a definite location in the nucleic acid molecule, and this should be an event of very low frequency. By direct determination, most mutations occur spontaneously with a frequency of less than one per million normal copies and some have been measured at frequencies as low as one per ten billion replications. For instance the clinically very important mutation of the tubercle bacillus to streptomycin resistance has a frequency of about one per ten billion normal bacteria. Such a rare event could never be detected except for the fact that in an environment rich in streptomycin the ten billion normal bacilli are killed, but the one resistant bacterium is able to multiply very happily. This process is called the selection of mutants by alteration of the environment and is the standard tool of the virologist interested in the genetics of his material. For instance, one can grow type 1 polio virus in monkey testicular tissue, a host cell that is not normal for this virus. Any mutant virus particles that are better fitted to multiply in this unaccustomed environment will eventually outgrow the parental virus type and may be obtained in pure culture. Li, Schaeffer and Nelson[4] who did this experiment, found that the isolated virus mutant was no longer virulent for monkeys by intracerebral inoculation.

If this is the correct picture of mutation, then it would seem reasonable that a second error of copying, occurring at the same site as the first, might restore the nucleic acid to its original form and so cause a reversal of the mutation. This is exactly what is observed in the case of single step mutations in both bacteria and viruses. For instance in the case of streptomycin resistant bacteria, mutation in the reverse direction to streptomycin sensitivity occurs with about the same frequency as the original mutation to resistance.

The situation is quite different in the case of multiple step mutations. For instance the acquisition of full penicillin resistance by staphylococci requires five separate mutations. Ordinarily it would be impossible for the organism to proceed systematically through this particular series of mutations. However, in the penicillin rich environment furnished by our physicians, each step in the sequence is of enormous value to the bacterium, so that its predecessor is eliminated and replaced by the more resistant mutant which then mutates to the next step in

the sequence. Once such a bacterial strain has achieved full penicillin resistance there is a negligible probability that it will be able to reverse each of the five mutational steps unless it is placed in a new environment in which *each* reverse step is advantageous. The net effect is that the acquisition of penicillin resistance by the staphylococcus is an irreversible process. The same principle applies to virus mutations. The classic 17D strain of yellow fever, developed by Theiler and used on an enormous scale as a living vaccine in man, was the end result of a long series of mutations selected by passage through several kinds of host cells. This strain has shown no tendency to revert to virulence for man.

Another potential source of genetic variability in populations is hybridization or Mendelian recombination. Until recent years it was generally believed that both bacteria and viruses were devoid of sexual activity but in the year 1946 clear cut evidence for hybridization of both kinds of organisms was published. Since that time hybridization has been demonstrated with a number of different viral strains, including the influenza viruses. The situation is at present somewhat confused in the case of hybridization in the poliomyelitis group, but at least two different laboratories are actively working on the problem and definitive information should be available in the near future. It is obviously too early to speculate on the possibility that hybridization of two virus strains of low virulence may produce a new strain of increased virulence, but I assume this possibility has been kept in mind by those working with live vaccines.

The most striking property of viruses and indeed the only property that is essential for their recognition is their ability to cause damage to their host. A virus that caused no disease would not be noticed. We may be hosts for many unknown viruses that we have no way of detecting. The multiplying virus, while tapping the host cell for necessary supplies of energy and raw materials, often disorganizes the economy of the host to the point of irreversible damage. If the host cell happens to be part of an essential structure such as a nerve, the virus will produce obvious disease. If, in contrast, the host cells are in the intestinal tract and expendable the virus may show no evidence of its sojourn, other than the persistent production of viral antibodies.

The impressive advances in our understanding of the polio and related viruses during recent years are largely the result of the discovery

by Enders, Weller and Robbins in 1949 that these viruses can multiply in cultures of non-nervous tissue. The use of tissue culture techniques has permitted the study of the multiplication of polio virus in suspensions of mammalian cells. For convenience the infectious process in a single host cell may be divided into four stages: adsorption, penetration, intracellular multiplication and the release of viral progeny. After completion of such a cycle of virus multiplication, the newly liberated virus particles adsorb to neighboring host cells and initiate a new cycle of virus growth. The time required to complete a single cycle of virus growth depends on the properties of the virus, host cell and on the environmental conditions. For the three types of polio virus growing in monkey kidney cells at 37 °C the average time required for one growth cycle is about four hours and the average yield is several hundred virus particles per infected host cell.

The first stage in the virus life cycle, adsorption of virus to host cell, is the stage most accessible to observation and control, and consequently is best understood. Adsorption is a highly specific chemical reaction. This specificity is a major factor in determining the susceptibility or resistance of a host cell to infection by a virus. As an example the kidney cells of capuchin monkeys do not adsorb type 2 polio virus whereas kidney cells of rhesus monkeys do adsorb type 2 virus and serve as satisfactory host cells. The capuchin monkeys are resistant to the intracerebral injection of type 2 virus and rhesus monkeys are susceptible,[5] so that this may reflect a species difference involving many types of cells. Mutation may so alter the surface structure of a virus that it can adsorb to new kinds of host cells, or lose its ability to adsorb to types of host cells that were previously susceptible. Such host range mutations are very common in viruses and may be the basis for the development of live virus vaccines.

Adsorption of the virus may be prevented in an entirely different manner by modification of the surface of the host cells either by mutation or by environmental influences. The chorioallantoic membrane of the chick embryo may be modified by treatment with an enzyme, so that the influenza virus will no longer adsorb and the embryo is rendered temporarily resistant to infection. In this case the enzymatic action is directly on the host cell receptor sites to which the virus would normally adsorb.

There are several examples of polysaccharides which will combine

with viruses and modify their ability to adsorb to the host cell. One well studied case is the normal inhibitor of influenza virus that is present in tissue fluids and secretions of man. This inhibitory polysaccharide is present in the mucin that bathes the respiratory epithelium and so would protect man from attack by the influenza viruses were it not for the unfortunate fact that the influenza virus possesses an enzyme that enables it to destroy the inhibitor. This enzyme is essential for the infectivity of the virus and a drug that would inactivate the viral enzyme might be an effective chemotherapeutic agent against influenza. Another example of a polysaccharide inhibitor has been described for Theiler's GDVII virus of mouse encephalomyelitis.[6, 7] This polysaccharide is found in the small intestine and stomach of mice but in no other organs. It neutralizes the infectivity of GDVII virus but not that of the similar TO virus. It is significant that although both viruses are highly virulent when injected by the intracerebral route, only the TO virus is able to multiply in the intestinal tract of mice.

Of all the methods of interference with the adsorption of virus to host cell, the most valuable is the use of specific anti-viral serum. In fact active and passive immunization procedures are the only effective ways available at present to protect man from attack by the smaller viruses in his environment. There is conclusive proof in the case of some viruses that homologous antibody does not kill the virus particle but merely interferes with adsorption to the host cell and with penetration. The evidence is that viral infectivity can be restored when the neutralized virus is treated with proteolytic enzymes that hydrolyze the antibody molecules. In certain systems the reaction between virus and antibody seems to be partially reversible, but only under conditions that do not pertain *in vivo*. The neutralization of the polio viruses by antibody seems to be irreversible. In human infections with polio virus, a viremia seems consistently to precede infection of the nervous system. It is during this period of viremia or prior to it, that virus may be neutralized by antibodies. The probability of a virus particle becoming neutralized before invading the nervous system is directly proportional to the concentration of antibody in the serum, so that both antibody titer and its duration are important considerations in active immunization.

The second step in the growth cycle of viruses involves the penetration of part or all of the virus particle through the host cell wall.

In the case of some bacterial viruses there is clear evidence that the virus nucleic acid penetrates but that the viral protein remains attached to the outside surface of the host cell, and plays no further role after penetration is completed. In the case of the influenza viruses the evidence indicates that the entire virus particle penetrates. Nothing is known about penetration of the poliomyelitis viruses. In the case of certain bacterial viruses, penetration involves an enzyme catalyzed reaction in which calcium ion plays an essential role. For these viruses citrate can serve as a chemoprophylactic agent. The possibility of chemical control of the penetration of mammalian viruses does not seem to have been considered seriously.

The third stage, intracellular virus multiplication, is still enshrouded in mystery. One feature that seems to be common to all the smaller viruses is the "eclipse period" during which it is impossible to recover infectious virus particles by breaking open the infected cells. This suggests that actively multiplying, vegetative virus is structurally different from mature virus particles that have ceased to multiply. It is assumed that during, or following penetration, the virus nucleic acid becomes separated from the virus protein. The virus nucleic acid proceeds to multiply, or the host cell makes replicate copies of the virus nucleic acid during the eclipse period. After many copies of the virus nucleic acid have been completed, they are coated with virus protein to form mature, infectious virus particles ready for liberation from the host cell. According to this picture, vegetative virus consists of virus nucleic acid plus all the metabolic machinery needed to make more copies of the nucleic acid. In contrast, mature virus consists of virus nucleic acid in a resting stage coated with a protective protein layer whose sole function is to aid the virus nucleic acid in its transfer from one host cell to another. It seems clear that there are many possibilities for chemical control of intracellular virus replication, but so far nothing of practical value has been developed for control of the smaller viruses. It should be emphasized that antibodies against viruses have no effect on virus multiplication within cells that are already infected. The only function of antibodies is to prevent infection by the neutralization of extracellular virus. It is for this reason that the serum therapy of virus diseases has been so notably ineffective.

The last stage in the life cycle of many but not all viruses involves destruction of the host cell and liberation of the mature virus particles

into the extracellular environment. In many cases this is an explosive event involving rupture of the cell wall and extrusion of the cell contents. In other cases the process appears to be more gradual and virus is liberated over a considerable period of time. With the polio viruses growing in monkey kidney tissue culture, degenerative changes become evident within two hours after infection. Then changes in the appearance of the cell wall occur, followed by rupture of the wall and release of the bulk of the virus progeny together with much of the cell cytoplasm about four hours after infection.

We have attempted to present an abbreviated description of the physical, chemical and biological properties of the smaller viruses. It seems clear that at the present time there is no prospect of effective chemoprophylaxis or chemotherapy of diseases due to these viruses, although in the long run this may become possible. The only practical method in sight for the large scale prevention of these virus diseases involves active immunization with virus vaccines. The effectiveness of this method depends on the maintenance of a sufficient level of circulating antibody to prevent virus particles from reaching their susceptible host cells. It should perhaps be emphasized that as far as the invading virus particle is concerned it makes absolutely no difference whether the neutralizing antibody that terminates its career was produced in response to a killed vaccine or a live virus vaccine. However, from the point of view of the scientist there are plenty of arguments both for and against each type of vaccine as we shall undoubtedly hear.

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